# This Month in *The Journal*

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## Optimized Southern Blot for Determining *C9orf72* Repeat Expansion Size

**Beck et al., page 345**

The association between *C9orf72* hexanucleotide repeat expansions and diseases such as frontaltemporal lobar degeneration (FLTD) and amyotrophic lateral sclerosis (ALS) has received noteworthy attention recently. However, defining the role of *C9orf72* expansions in neurodegeneration is complicated because it is difficult to quantify the number of repeats and thus correlations between repeat expansion number and phenotype are often ambiguous. To sort out this puzzle, Beck et al. used repeat-primed PCR and an optimized Southern blot to determine the frequency of large repeats in disease and control cohorts. Across six neurodegenerative cohorts, large repeat expansions accounted for 7.5% of FLTD, 1.2% of Alzheimer disease, 8.1% of ALS, 0.2% of sporadic Creutzfeldt-Jakob disease, 1.7% of Huntington-disease-like syndrome, and 2% of other neurodegenerative diseases. Interestingly, there was a correlation between the size of the expansion and the age of onset, but not between expansion size and diagnostic group. These expansions were also detected in 1/691 healthy individuals and were associated with a risk haplotype designated by rs3849942A. By ruling out a founder effect, Beck and colleagues suggest that this haplotype might confer an increased risk of mutation. Because of the high number of carriers and the frequency of expansions in several neurodegenerative diseases, the utility of a reliable method for quantifying expansion size, such as the optimized Southern blot used in this work, will be increasingly important as a diagnostic and investigative tool.

## A SNP Slows Migration

**Pu et al., page 366**

Genome-wide association studies (GWASs) have provided hints about genes that might be involved in certain diseases and complex traits. However, the impact of many significant SNPs remains speculative and requires characterization for clarifying their biological function. In this study, Pu et al. followed up on one such SNP, rs3825807, which they identified in a population-based study of atherosclerosis and which has also recently been associated, by GWAS, with coronary artery disease. This SNP is inversely associated with atherosclerosis and changes an amino acid from a serine (the A allele) to a proline (the G allele) in the prodomain of the protease ADAMTS7, which cleaves thrombospondin-5, a protein that is produced by vascular smooth muscle cells (VSMCs) and that inhibits their own migration. Cells expressing the G allele were not as efficient in cleaving thrombospondin-5, had reduced accumulation of prodomain processing, and had a reduced migratory ability. This is consistent with the idea that the serine-to-proline substitution in the prodomain might interfere with ADAMTS7 maturation and thus lead to reduced function in the variant encoded by the G allele. Although the specific mechanism by which reduced VSMC migration protects an individual from atherosclerosis remains unclear, this study provides insight into the more subtle functional differences that can arise from nonsynonymous variants and serves as an example of using specific GWAS data as a starting point for in vivo characterization.

## Zooming In on CNVs

**Chen et al., page 375**

The neurexin proteins (NRXN1, NRXN2, and NRXN3) play key roles in synapse formation and neurotransmission. It is perhaps not surprising, then, that *NRXN1*-affecting microdeletions, which occur with high frequency, confer risk of neurodevelopmental abnormalities, including autism spectrum disorder and schizophrenia. Owing to the variability of the size and location of the deletions, however, the mechanism(s) that target this region have remained unknown. In this study, Chen et al. analyzed the breakpoints of over 30 *NRXN1* deletions and propose that the presence of short inverted repeats predisposes this region to genomic instability and thus renders it a so-called deletion hotspot. Although the authors' analysis was limited to deletions affecting *NRXN1*, these results should be of considerable interest to investigators who are interested in better understanding the mechanisms that underlie complex patterns of copy-number variation and their related genomic disorders.

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## A New Look at the Y

**Mendez et al., page 454**

Efforts to uncover the origins of anatomically modern humans have relied, in large part, on analyses of mtDNA and the nonrecombining portion of the Y chromosome. These studies have led to a well-accepted model that places the origin of our most recent common ancestors in sub-Saharan Africa. Unsatisfyingly, however, timing estimates from the female- and male-specific sequences differ by ~100,000 years. Although several potential explanations have been put forth for the more ancient history of mtDNA lineages, this discrepancy has unsettled many investigators. Now, Mendez et al. report on the serendipitous discovery of a Y chromosome that harbors the ancestral state of all known SNPs. This finding led them to add a new root to the Y chromosome phylogenetic tree, and they termed this lineage A00. Armed with these findings, the authors developed new estimates that suggest that the Y lineage is much older than any previously identified mtDNA lineages. The authors' work is sure to send many investigators back into the lab, where they will need to consider not only new models for Y chromosome diversity, perhaps by drawing upon knowledge of autosomal diversity, but also a reassessment of exactly who our earliest ancestors were.

## ONECUT Ahead

**Funnell et al., page 460**

Hemophilia B, also known as the "royal disease" for its prevalence in descendants of England's Queen Victoria, is known to arise from mutations in coagulation factor IX (*F9*). Although over 20 different mutations have been identified in the *F9* promoter region, the molecular cause of this blood disease has remained a mystery for many affected individuals. In this issue, Funnell et al. demonstrate that a cluster of mutations in the *F9* promoter disrupts binding of transcription factors ONECUT1 (also known as HNF6) and ONECUT2. Mice lacking these transcription factors died in utero, owing to developmental defects. The authors showed that *F9* expression was markedly reduced in these embryos; notably, other liver-specific genes were expressed at near normal levels, indicating that the reduced *F9* levels do not stem from a general failure in liver development. The ONECUT proteins join HNF4 and C/EBP$\alpha$ as known regulators of *F9* expression, thus expanding our knowledge of this liver-restricted transcriptional program. For those with an appreciation of history, these findings also solve a puzzle that has stymied geneticists for many years.